

Case Report

A Case of Metastatic Adrenocortical Carcinoma Diagnosed with Steroidogenic Factor-1 in a Sprague-Dawley Rat

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Abstract: This report describes the morphological and immunohistochemical characteristics of an adrenocortical carcinoma with distant metastasis in a Sprague-Dawley rat. Macroscopically, a single large mass was observed in the adrenal gland, and multiple nodules were noted in the lung, liver and thyroid. Histologically, the adrenal tumor consisted of a solid growth of eosinophilic round cells with nuclear atypia. Vascular invasion was present, and multiple metastatic lesions were also observed in the lungs, liver, and mediastinal lymph nodes. Immunohistochemically, the nuclei of these tumor cells were positive for Steroidogenic Factor-1 (SF-1). In the thyroid, tumor cells histologically resembling adrenal cells were immunohistochemically negative for SF-1 but positive for calcitonin; thus the lesion was diagnosed as thyroid C-cell carcinoma. From these results, the present case was diagnosed as adrenocortical carcinoma with distant metastases. SF-1 could be a valuable marker for the differential diagnosis of adrenocortical tumors versus other endocrine tumors such as C-cell carcinoma. (DOI: 10.1293/tox.26.319; J Toxicol Pathol 2013; 26: 319–323)

Key words: adrenocortical carcinoma, immunohistochemistry, Steroidogenic Factor-1

Spontaneous adrenocortical carcinomas occur at a relatively low frequency in most rat strains with the exception of some specific strains including Osborne-Mendel rats and highly inbred lines of Wistar rats^{1,2}. In Sprague-Dawley rats, the incidence of adrenocortical carcinomas is reported to be around 0–1.2%^{3–6}. In tumor-prone rats, metastasis to distant organs such as regional lymph nodes and the lungs has been described^{1,2}; however, to our knowledge, there have been no reports regarding adrenocortical carcinoma with distant metastasis in Sprague-Dawley rats^{3–5}. We encountered a case of adrenocortical carcinoma with metastasis to the lungs, liver and mediastinal lymph node in an aged female Sprague-Dawley rat. Because this animal coincidentally had a thyroid C-cell carcinoma, which consisted of a solid proliferation of round tumor cells resembling adrenocortical carcinoma cells, appropriate differential diagnosis especially for the metastatic sites was needed. Here, we report the histological features of this rare case of metastatic adrenocortical carcinoma and also demonstrate the utility of Steroidogenic Factor-1 (SF-1), a nuclear receptor with critical roles in steroidogenesis⁷ as an immunohistochemical marker for adrenocortical tumors.

A thirteen-week-old female Sprague-Dawley rat was

purchased from Charles River Laboratories Japan (Hino, Japan), housed in a metal cage in an animal room at Takeda Rabics Limited (Yamaguchi, Japan) with a temperature of 20–26°C, 40–80% relative humidity and a 12-hour light/dark cycle and fed a commercial diet (CR-LPF: Oriental Yeast Co., Ltd., Tokyo, Japan) and tap water *ad libitum*. At 108 weeks of age, the animal was transported from Takeda Rabics Limited to the Shonan Research Center of Takeda Pharmaceutical Company Limited (Kanagawa, Japan) and was immediately sacrificed by exsanguination from the abdominal aorta under inhalation anesthesia with isoflurane. The experimental procedures were approved by the Institutional Animal Care and Use Committees of Takeda Pharmaceutical Company Limited. There were no clinical signs before necropsy. At necropsy, a dark red mass approximately 30 × 25 × 20 mm in diameter was observed in the left adrenal gland (Fig. 1A); however, the presence of the contralateral (right) adrenal gland was not confirmed macroscopically. In addition, multiple yellow or white nodules less than 10 mm in diameter were found in all lobes of the lungs (Fig. 1B), two white nodules approximately 10 mm and 1 mm in diameter were found in the left lateral lobe and the caudal part of the caudate lobe of the liver, and a white nodule approximately 5 mm in diameter were found in the right side of the thyroid. The mediastinal lymph nodes were enlarged with dark red discoloration (Fig. 1B). Furthermore, a dark red focus was observed in the pituitary gland. There were no remarkable findings in the other organs and tissues. All of the gross lesions mentioned above were fixed in 10 vol% neutral buffered formalin, embedded in paraffin,

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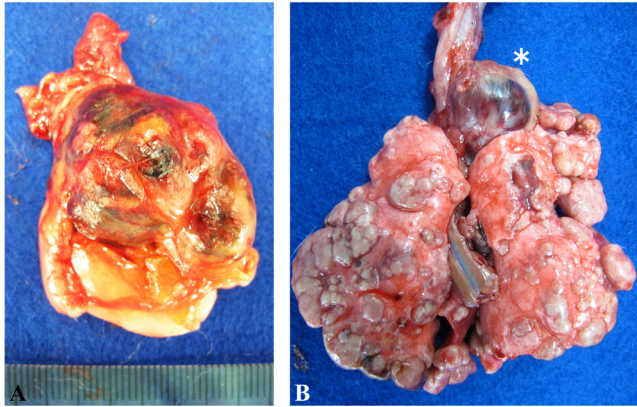


Fig. 1. Macroscopic findings of masses in the adrenal gland and lung. (A) Appearance of the adrenal mass. A dark red mass approximately $30 \times 25 \times 20$ mm in diameter was observed in the left adrenal gland. (B) The dorsal aspect of the lung and mediastinal lymph nodes. Multifocal pulmonary nodules less than 10 mm in diameter were observed in all lobes, and enlargement of mediastinal lymph nodes with dark red discoloration (*) was observed.

sectioned and stained with hematoxylin and eosin (H.E.). For the differential diagnosis, sequential sections for each lesion except the pituitary lesion were immunohistochemically stained with anti-human Steroidogenic Factor-1 mouse monoclonal antibody (diluted 1:1000, clone: N1665, Perseus Proteomics, Tokyo, Japan) and anti-human calcitonin rabbit polyclonal antibody (diluted 1:100, GeneTex, Inc., Los Angeles, CA, U.S.A.), and the pituitary was immunostained with anti-rat LH rabbit polyclonal antibody (diluted 1:5000, Accurate Chemical & Scientific Corp., Westbury, NY, U.S.A.) and anti-human ACTH mouse monoclonal antibody (diluted 1:1000, clone: 56, Novocastra Laboratories Ltd., Newcastle, U.K.).

Microscopically, the adrenal mass consisted of a solid proliferation of tumor cells, and the remaining cortical tissue was significantly distorted by these tumor cells. The tumor cells were round, possessed abundant eosinophilic cytoplasm with occasional vacuolation and were arranged in nests or trabeculae, which were separated by a fibrovascular stroma. The nuclei were round and had a low chromatin density and distinct single nucleoli. Anisokaryosis and mitotic figures were frequently observed (Fig. 2A), and massive necrosis and multifocal hemorrhage were also seen within the tumor. As findings that indicated malignancy, local and vascular invasions by the tumor cells were observed (Fig. 2B); therefore, the adrenal tumor was diagnosed as adrenocortical carcinoma and was subclassified into the well-differentiated type due to the degree of differentiation of the tumor cells.

The histological features of the nodules in the lungs and liver and enlarged mediastinal lymph node were very similar to those in the adrenal mass. That is to say, the lesions consisted of solid proliferations of round tumor cells with abundant eosinophilic cytoplasm, occasionally vacu-

olated, and round nuclei with nuclear atypia (Fig. 2C–E). Massive necrosis and multifocal hemorrhage were also frequently observed within these lesions. The thyroid mass also consisted of a solid proliferation of round tumor cells with abundant eosinophilic cytoplasm and round nuclei with a lower chromatin density; however, the cytoplasm was slightly pale, vacuolation was rare, and the nucleoli appeared to be indistinct compared with those in the adrenal tumor. Normal follicles were often seen within the tumor tissue (Fig. 2F). The cellular morphology and presence of normal follicles indicated that the origin of the tumor cells was thyroid C-cells, and since local invasion of tumor cells to the adjacent tissues was partly evident, the thyroid tumor was considered to be a C-cell carcinoma.

Immunohistochemically, almost all nuclei of the normal adrenocortical cells as well as the tumor cells in the adrenal gland, lungs, liver and mediastinal lymph nodes were positive for SF-1 (Fig. 3A–D), indicating that these tumors were derived from steroidogenic cells. Since there were no proliferative lesions in the steroidogenic tissues other than the adrenal gland, the lesions in the lungs, liver and mediastinal lymph nodes were determined to be metastases of the adrenocortical carcinoma. In addition, all the tumor cells in these tissues were negative for calcitonin. On the other hand, all the nuclei of the thyroid tumor cells were negative for SF-1 (Fig. 3E), and a positive reaction for calcitonin in the cytoplasm of the tumor cells was confirmed (Fig. 3F). Hence, the thyroid tumor was definitely diagnosed as C-cell carcinoma.

To clarify whether there was any morphological change in the corticotrophs or not, we also examined the pituitary histopathologically. Two instances of focal hyperplasia and one adenoma, both consisting of amphophilic cells, were observed in the pituitary; however, all these cells were positively immunostained with LH antibody, and thus, they were diagnosed as gonadotroph hyperplasia and adenoma. Meanwhile, immunohistochemical staining with ACTH antibody revealed no obvious changes in the distribution and density of the corticotrophs in this animal.

Based on the results described above, the present case was diagnosed as an adrenocortical carcinoma with metastases to the lungs, liver and mediastinal lymph nodes and a thyroid C-cell carcinoma. It was unclear whether the adrenal carcinoma was functional or not, since hormone measurements for corticosterone and ACTH were not conducted for this animal. However, it was suggested that the plasma ACTH levels remained normal, since histopathological examination of the pituitary gland showed no obvious changes in the corticotrophs. Both adrenocortical and thyroid C-cell carcinomas are relatively rare in rats, and there is no report describing metastasis of adrenocortical carcinoma in Sprague Dawley rats^{3–5}. Therefore, the present case is considered to be notably rare as a spontaneous tumor in Sprague-Dawley rats. Because the histological features of adrenal and thyroid tumors often closely resembled each other, immunohistochemical staining with SF-1 was enormously useful, especially for diagnosis of the lesions at the

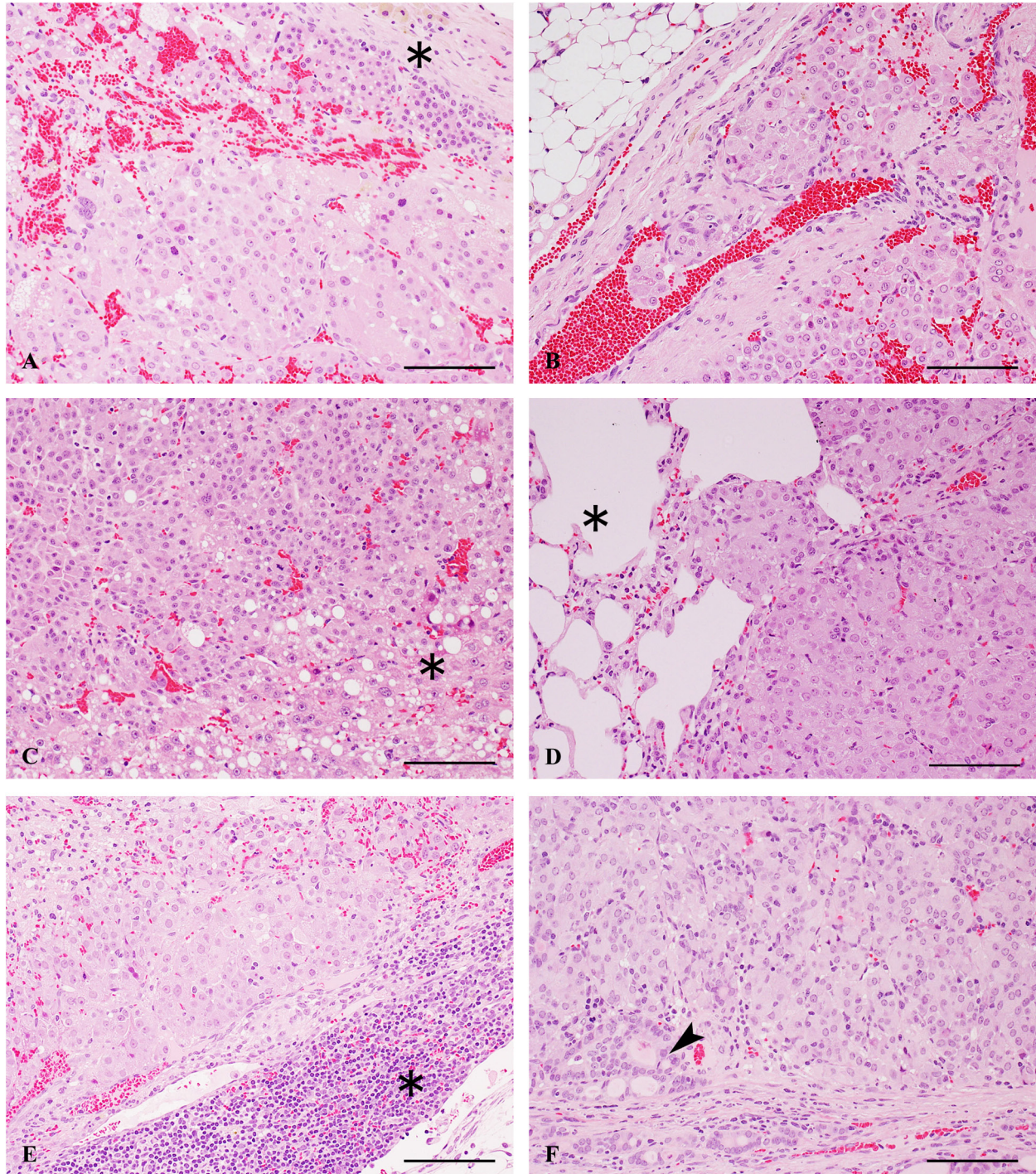


Fig. 2. Histological features of tumors in the adrenal gland (A and B), liver (C), lung (D), mediastinal lymph nodes (E) and thyroid (F). (A) There were an adrenal mass that consisted of a solid proliferation of round tumor cells with abundant eosinophilic cytoplasm, occasionally vacuolated, and round nuclei. The normal adrenal cortex (*) was distorted by the tumor tissue. (B) Vascular invasion of tumor cells was observed. (C, D, E) Tumor cells similar to those in the adrenal tumor proliferated in the liver, lung and mediastinal lymph nodes, respectively. Normal hepatic, pulmonary and lymphatic tissue was observed around the tumor tissue (*). (F) The thyroid mass consisted of a solid proliferation of round tumor cells with abundant eosinophilic but slightly pale cytoplasm, and round nuclei. Normal follicles were often seen within the tumor (arrowhead). Normal thyroid tissue was distorted by the tumor tissue. H.E. stain. Bar = 100 μ m.

metastatic sites. SF-1 is a nuclear receptor with critical roles in steroidogenic tissues as a transcriptional factor⁷ that is distributed in not only the steroidogenic organs such as the

gonads and adrenal cortex including the X-zone and subcapsular polygonal (type B) cells in mice⁷⁻¹⁰ but also in the ventromedial hypothalamic nucleus^{11,12} and pituitary gonado-

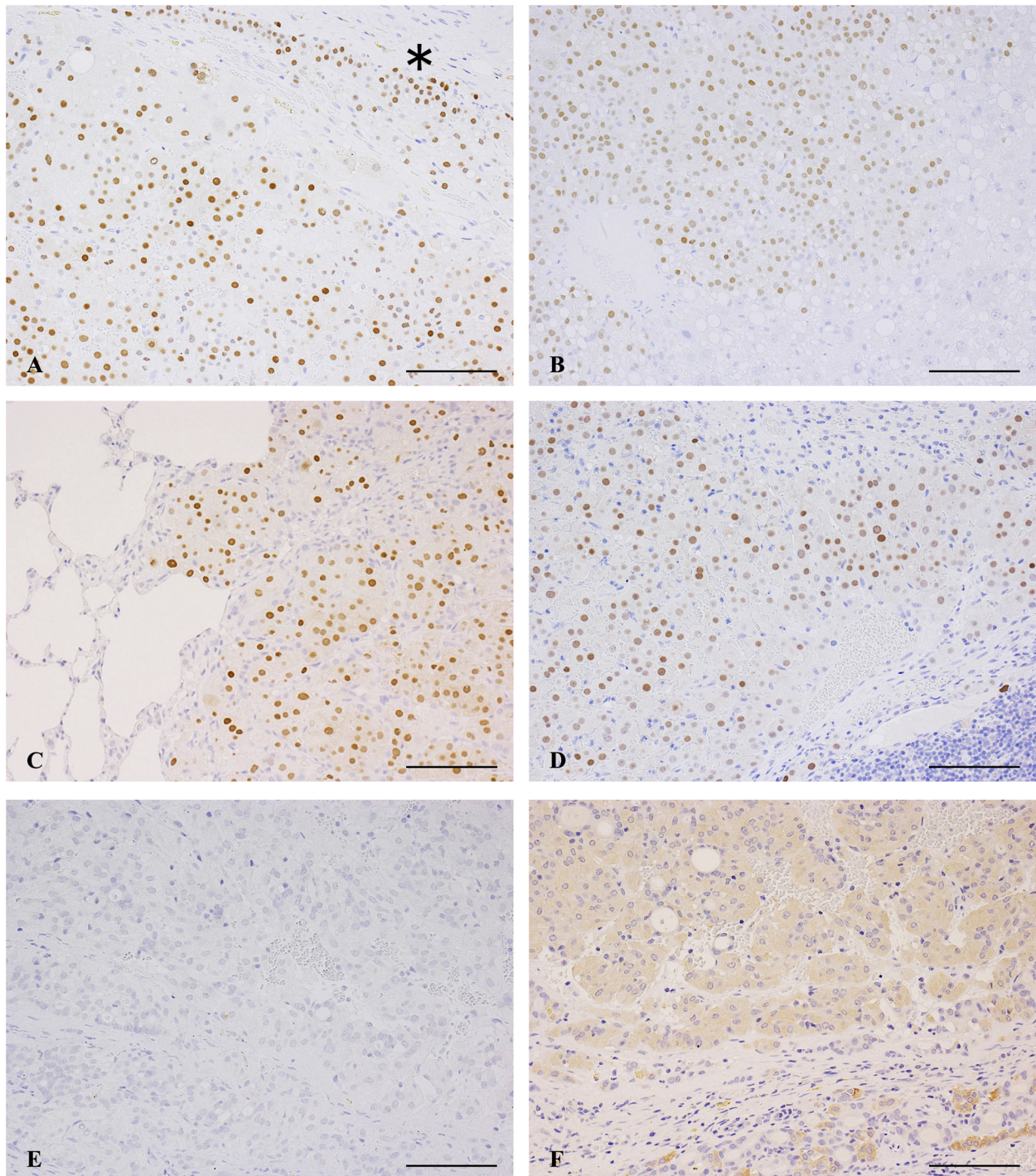


Fig. 3. Immunohistochemistry for SF-1 in the tumors in the adrenal gland (A), liver (B), lung (C), mediastinal lymph nodes (D) and thyroid (E), and for calcitonin in the tumor in the thyroid (F). The regions of the pictures are approximately consistent with those in H.E. sections shown in Fig. 2. (A, B, C, D) Almost all the nuclei of the tumor cells in the adrenal gland, liver, lung and mediastinal lymph nodes were positive for SF-1 with distorted normal adrenocortical cells (A, *). (E) All nuclei of the tumor cells in the thyroid were negative for SF-1. (F) The cytoplasm of the tumor cells in the thyroid was positive for calcitonin, as in the normal C-cells in the distorted thyroid tissue. Bar = 100 μ m.

trope¹³. SF-1 is a major regulator of cholesterol metabolism in steroidogenic cells, as it stimulates the expression of nearly all the factors involved in cholesterol mobilization and steroid hormone biosynthesis⁷. Recently in humans, SF-1

was revealed to be a highly valuable immunohistochemical marker for determination of the adrenocortical origin of an adrenal mass with high sensitivity and specificity¹⁴, and its expression is of stage-independent prognostic value in

patients with adrenocortical carcinoma¹⁴. Currently, there is no reliable immunohistochemical marker for adrenocortical tumors in rats; however, the present results demonstrate that SF-1 is also useful in the case of rats. Adrenocortical tumors have been induced experimentally with variety of agents including estrogens¹⁵, irradiation^{16–18}, certain chlorinated hydrocarbons^{19–21} and benzenes²². Therefore, proliferative changes in the adrenal cortex might be induced in rat carcinogenicity studies, and in some cases, the differential diagnosis of various types of endocrine tumors including thyroid C-cell, parathyroid and islet cell tumors, pheochromocytoma and metastatic hepatocellular tumor may be required due to the histological similarities of these tumors and the morphological heterogeneity of adrenocortical tumors^{2,14}. Although further investigation using the other type of tumors including poorly differentiated or anaplastic types may be necessary, immunohistochemistry for SF-1 is considered to be a valuable marker for the differential diagnosis of adrenocortical tumors versus other tumors such as thyroid C-cell carcinoma in rat carcinogenicity studies.

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